

## Uterine bacterial infection during postpartum delays the recrudescence of the reproductive traits in dairy cows

Faisal Omer Ahmed<sup>1</sup>, Adil Salim Elsheikh<sup>1,2</sup>

<sup>1</sup>Department of Reproduction and obstetrics, Faculty of Veterinary Medicine, University of Khartoum, Shambat, Sudan

<sup>2</sup>Department of Applied Medical Sciences, Community college, Najran University, Saudi Arabia

Email: [adilelgarrai@yahoo.com](mailto:adilelgarrai@yahoo.com)

**Abstract:** This study was designed to determine the intensity of bacterial load in the uteri of postpartum (PP) dairy cows and the effects of this uterine bacterial infection on some of their reproductive traits. Six reproductive traits were evaluated. These traits were uterine involution (UI), appearance of the first dominant follicle (DF), recrudescence of the first oestrus (FO), length of the days open (DO), calving interval (CI) and the rate of service per conception (RS). Uterine endometrial swabs were collected on day 5 PP from 130 dairy cows and were cultured within 2 hours of collection onto blood agar and MacConkey agar media. The uteri of 120 cows (93%) were found infected. From the total infected cows 40 cows were used to evaluate the effect of bacterial uterine infection on their reproductive performance. Twenty cows were severely infected and the remaining cows (20 cows) were mildly infected. The result of this experiment showed that dairy cows that suffered severe uterine bacterial infection had a significantly ( $P < 0.001$ ) extended uterine involution period, long time for the appearance of the first DF and the FO, the length of DO and CI compared to the dairy cows that suffered mild uterine bacterial infection. Moreover, the dairy cows which suffered severe uterine bacterial infection had a significantly ( $P < 0.001$ ) increased rate of service per conception compared to the cows with mild uterine bacterial infection. It is concluded that, the reduced reproductive efficiency of cross-bred dairy cows in the Sudan is likely to be due to early PP uterine bacterial infection.

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### 1. Introduction

The long PP period in cross-bred dairy cows in the Sudan is the major problem that limits the improvement of their reproductive efficiency (Ahmed and Elshiekh, 2004; Ahmed and Elshiekh, 2005, Abdallah, 2006), which was found to be due to uterine bacterial infection; bacterial products or the associated inflammation that reduces FSH concentrations and suppresses LH release (Opsomer et al., 2000). Consequently, this perturbs PP ovarian activity and functions, which causes cystic ovary in dairy cattle. The initiation of PP follicular wave, appearance of the first DF, recrudescence of the FO, improvement of conception rate, DO and CI are influenced by several PP factors (Noakes et al., 1999). A complex relationship exists between factors influencing uterine health and disease in the PP (Gilbert, 1992; Nebel, 1999). The reproductive performance of dairy cows after the voluntary waiting period is highly related to the health status of the uterus after calving (Dijkhuizen and Stelwagen, 1995; Ferguson and Galligan, 2000). The PP uterine infection occurs in the cows as sequellae to retention of the fetal membranes, dystocia and descending infection (Fredriksson et al., 1988). Cows with

infected uteri are known to have an increased rate of services per conception (Heuwieser et al., 2000). It has been reported that 90% of bovine uteri are infected up to day 15, 78% up to day 30, 50% up to day 45 and 9% up to day 60 PP (Leslie, 1983). The abnormal puerperium adversely affects uterine defense mechanism and prolongs the time taken for complete UI (Butt et al., 1991; Lander chacin et al., 1991). The current study was conducted to determine the common pathogens during early PP, percentage of dairy cows having uterine bacterial infection and to evaluate the effects of this uterine bacterial infection on UI, appearance of the first DF, recrudescence of the FO, DO, rate of service per conception and CI in cross-bred dairy cows in the Sudan.

### 2. Materials and methods

#### 2.1. Animals

The study was conducted on 130 cross-bred dairy cows (Friesian × Kenana) between 4 to 7 years old. Their BCS is between 2.5 to 3.5 according to the five-scale point system outlined by Wildman et al. (1982). According to this scale, emaciated cows are

scored 1, thin cows 2, average cows 3, fat cows 4 and obese cows are scored 5.

## 2.2. Uterine involution (UI)

It was determined by rectal palpation every other day after parturition till complete involution. The uterus was described to be involuted when the size of the uterine horn was equal two fingers and the uterine body was palpated in the pelvic cavity (Arthur et al., 1998). The complete uterine involution was assumed when the animal showed the first PP heat.

## 2.3. Postpartum dominant follicles detection (DF)

The PP follicular waves were checked manually by rectal palpation at two days interval until the first PPDF was detected (Duffy et al., 2000; Elsheikh and Ahmed, 2004).

## 2.4. Heat detection

All the cows in the herd were checked for oestrus signs by visual observations by well trained herd-men three times a day early in the morning at 7:00 am, in the mid day at 12:00 and at 7:00 pm for at least 30 minutes. The cows were recorded in heat when it becomes restless, licks the perineum of other cows, jumps on other cows, allow other cows or bull to mount her, it bellows and there is a transparent clear vaginal mucus discharge. The cow was considered in a full response when it stands to be mounted by the bull and mating was completed (Arthur et al., 1998; Elsheikh and Ahmed, 2004).

## 2.5. Days open (DO)

It has been done by calculating the interval in days from calving to the subsequent effective service date of those cows that conceive (Arthur et al., 1998; Elsheikh and Ahmed, 2004).

## 2.6. Calving interval (CI)

This parameter was carried out according to Arthur et al., (1998), Bath et al., (1985) and Elsheikh and Ahmed (2004) where the CI is the duration between two consecutive calving.

## 2.7. Early PP uterine swabs collection and bacteriology

A transcervical guarded sterile disposable swabs were collected from the endometrium of each cow on day 5 PP (Noakes et al., 1989 and Sheldon, et al., 2004). The swabs were transferred to sterile test tube and were cultured within 2 hours of collection. The swabs were cultured aerobically in pre-equilibrated sheep blood agar and on MacConkey agar and incubated at 37°C over-night. Identification of bacteria was based on the characteristic of colony, gram-stain and morphology (Barrow and Feltham, 1993). Bacterial growth on the cultured plates was scored semi-quantitatively depending on the number of bacterial colonies detected on the plate: 0: no growth; 1 < 10 colonies, 2,10 to 100 colonies, 3, 100

to 500 colonies and 4 > 500 colonies (Noakes et al., 1999).

## 2.8. Experimental design

**Experiment I:** This experiment was carried out to determine the percentage of uterine bacterial infection during early PP among 130 cross-bred dairy cows. The bacterial growth was graded into three grades 0: no growth, less than 100: mild infection and more than 100: severe infection (Noakes et al., 1989; Sheldon et al., 2004). Furthermore, the bacteria were identified and their percentage was recorded.

**Experiment II:** This experiment was carried out to monitor the effects of uterine bacterial infection during early PP on the following reproductive traits in cross-bred dairy cows. These parameters were UI, appearance of the first DF, resumption of the FO, DO, rate of service per conception and CI. From the total infected cows recorded in experiment I, 40 cows were used to evaluate the effects of bacterial uterine infection on reproductive performance of the dairy cows. Twenty cows were severely infected (Group A) and the other 20 cows were mild infected (Group B). The six parameters mentioned above were studied to assess the PP reproductive efficiency of the selected cows.

## 2.9. Statistical analysis:

The results were statistically evaluated by ANOVA followed by Fisher's protect least significant difference (PLSD). In a one factorial design using Stat View analytical computer package version 4.01. Significant differences at  $P < 0.001$  were considered.

## 3. RESULTS

### 3.1. Types and percentage of bacterial infection

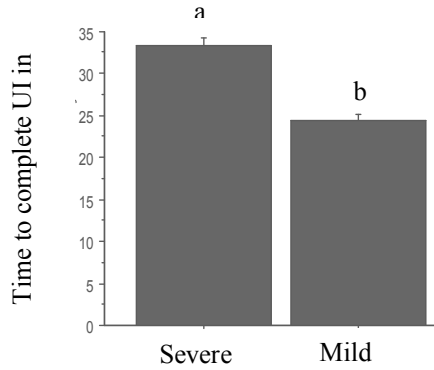
The uteri of 93% of the candidate dairy cows (120 cows) in this study were found infected during early PP. Only 7% of the employed dairy cows (10 cows) were found none infected. From the infected cows, 50% suffered severe uterine puerperal infection (60 cows). The remaining cows 50% (60 cows) suffered mild uterine bacterial infection. The common pathogenic bacteria isolated in this study from the endometrium of the candidate dairy cows during early PP were *Staphylococcus* spp (36%), *Streptococcus* spp (31%), *E coli* (24%) and *Pasteurella multucida* (9%).

### 3.2. The effects of uterine bacterial infection on reproductive traits:

#### 3.2.1 UI

The results of this experiment showed that the time taken for UI in dairy cows was significantly ( $P < 0.001$ ) influenced by uterine puerperal infection. Dairy cows suffered severe uterine puerperal infection had a prolonged time for uterine involution by about 10 days as compared to the cows suffered mild uterine puerperal infection. The mean length of the

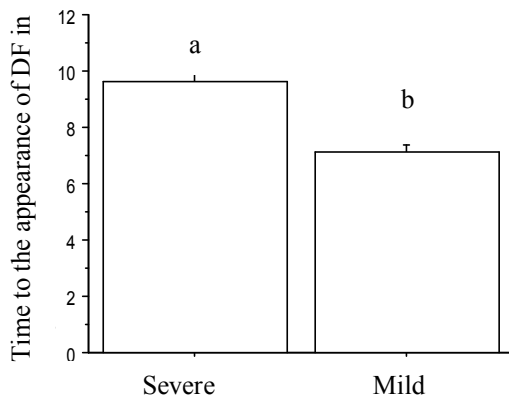
time taken for uterine involution in the dairy cows suffered severe uterine bacterial infection was  $33.40 \pm 0.90$  days. This period is longer than that of the dairy cows ( $23.70 \pm 0.60$  days) suffered mild uterine bacterial infection (Figure 1).



**Fig. 1.** The influence of uterine bacterial infection during early PP on the time taken to complete uterine involution in dairy cows (<sup>a,b</sup>  $P < 0.001$ ).

**3.2.2. First dominant follicle (DF)**

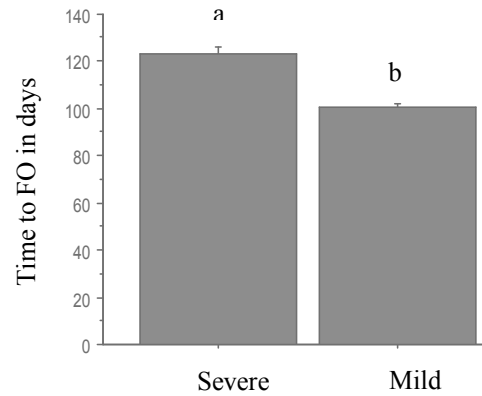
A severe PP uterine bacterial infection in dairy cows significantly ( $P < 0.001$ ) delayed the time taken for appearance of the first DF as compared to dairy cows suffered mild PP uterine bacterial infection. The mean length of the time taken for the appearances of the first DF in the dairy cows suffered severe uterine bacterial infection was  $9.60 \pm 0.40$  days. This value was longer than the time taken for the appearance of the first DF in dairy cows suffered mild uterine bacterial infection ( $7.10 \pm 0.30$  days) (Figure, 2).



**Fig. 2.** The influence of uterine bacterial infection on the appearance of the first DF during early PP in dairy cows (<sup>a,b</sup>  $P < 0.001$ ).

**3.2.3. Resumption of the first oestrus (FO)**

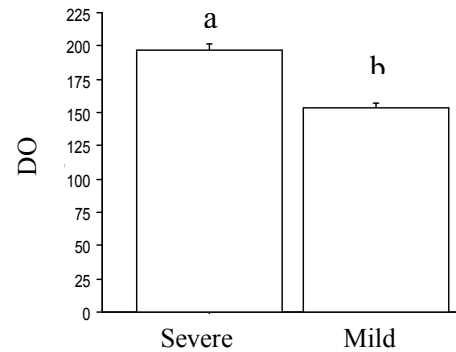
As shown in figure (3) the severe PP uterine bacterial infection in the dairy cows, significantly ( $P < 0.001$ ) extended the time taken for resumption of the FO compared to the dairy cows suffered mild PP uterine bacterial infection. The mean length of the period taken for resumption of the FO in dairy cows suffered a severe PP uterine bacterial infection was  $133.90 \pm 4.40$  days. This value was longer than that of the dairy cows with mild PP uterine bacterial infection ( $99.10 \pm 1.60$  days).



**Fig. 3.** The influence of uterine bacterial infection during early PP on the appearance of the FO in dairy cows (<sup>a,b</sup>  $P < 0.001$ ).

**3.2.4. Days open (DO)**

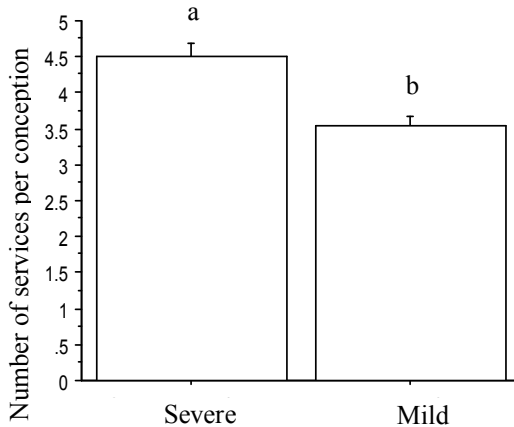
As showed in figure (4) the severe PP uterine bacterial infection in dairy cows, significantly ( $P < 0.001$ ) in the DO compared to the dairy cows suffered mild PP uterine bacterial infection. The severe uterine bacterial infection prolonged the DO by 79 days. The mean length of the DO of the dairy cows with severe uterine bacterial infection was  $212.40 \pm 8.20$  days. This value was longer than that of the dairy cows with mild uterine bacterial infection ( $133.80 \pm 4.90$  days).



**Fig.4.** The influence of uterine bacterial infection during early PP on the length of the DO in dairy cows (<sup>a,b</sup>  $P < 0.001$ ).

### 3.2.5. The rate of service per conception:

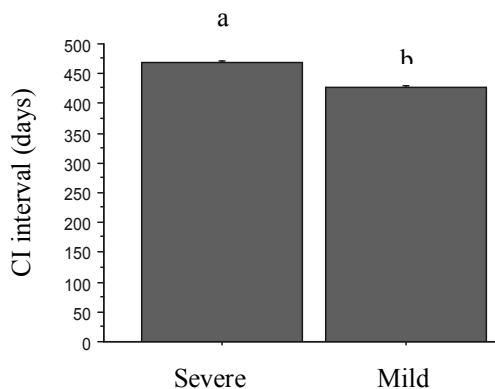
As showed in figure (5) the mean rate of service per conception for the dairy cows with sever PP uterine bacterial infection was  $4.50 \pm 0.20$  which was significantly higher ( $P < 0.001$ ) than that of the dairy cows with mild uterine bacterial infection ( $2.70 \pm 0.20$ ).



**Fig.5.** The influence of uterine bacterial infection during early PP on the rate of service per conception in cross-bred dairy cows (<sup>a,b</sup>  $P < 0.001$ ).

### 3.2.6. Calving interval (CI)

Figure (6) showed that the CI of the dairy cows with a sever PP uterine bacterial infection was significantly ( $P < 0.001$ ) longer compared to that of the dairy cows with mild PP uterine bacterial infection. The sever PP uterine bacterial infection in the dairy cows increased the CI by 75 days. The mean lengths of the CI of the dairy cows with sever PP uterine bacterial infection was  $482.50 \pm 9.00$  days and the CI of the dairy cows with mild PP uterine bacterial infection was  $407.10 \pm 4.80$  days.



**Fig.6.** The influence of uterine bacterial infection during early PP on the length of the CI in dairy cows (<sup>a,b</sup>  $P < 0.001$ ).

## 4. Discussion

This study confirms that, the reduced PP reproductive efficiency of dairy cows in the Sudan is mainly due to uterine bacterial infection. Forty percent of dairy cows were diagnosed with and treated for PP uterine bacterial infection (Leslie, 1983). In particular 90% of bovine uteri are infected up to day 15, 78% up to day 30, 50% up to day 45 and 9% up to day 60 PP (Sheldon et al., 2002; Sheldon et al., 2003, Sheldon et al, 2006; Foldi et al., 2006). This PP uterine bacterial infection delays UI, initiation of the first follicular wave, appearance of the first DF and the occurrence of the FO. Consequently, it prolongs the DO, increases the rate of service per conception at subsequent breeding and increases the CI (Bartlett *et al.*, 1986, Huszenicza et al., 1999). The above findings agree with the findings of this study in which 93% of uteri of the PP dairy cows were found infected. The major pathogenic bacteria isolated from uteri of the PP dairy cows were *Staphellococcus* spp, *Streptococcus* spp, *Pasteurella* *moltucida* and *E. Coli*. The uterine bacterial infection during early PP in the dairy cows is presumably due to poor sanitation and hygiene during normal delivery, dystocia or retention of the fetal membranes. In addition, contamination of the genital tract of the cows could be via the descending bacterial infection. Uterine bacterial infection, bacterial products or the associated inflammation reduce pituitary FSH, suppress LH release and perturb PP ovarian follicular growth and function, which delays the ovulation in dairy cattle (Opsomer *et al.*, 2000). The pathogens found in this study are similar to the pathogens reported in several studies that were reviewed elsewhere (Stevenson and Call, 1998). The presence of these pathogenic bacteria in the uterus during this critical period cause endometritis and induces histological lesions on the endometrium. It also delays the UI, initiation of follicular waves, the recrudescence of the FO and it perturbs embryo implantation. The effect of uterine bacterial contamination during early PP depends on the number and virulence of the organisms present as well as the condition of the uterus and its inherent defense mechanisms (Huszenicza et al., 2005). Dairy cows with mild uterine bacterial infection during early PP suffered less than the dairy cows with sever PP uterine bacterial infection. Ninety three percent of the cows in this study had bacterial infection during early PP. The cows that suffered severe PP uterine bacterial infection had a longer time taken to UI than the dairy cows that suffered from mild PP uterine bacterial infection. This can be attributed to the sever endometritis that occurred during early PPP which led to the damage of the endomerium and suppressed  $PGF_{2\alpha}$  which is essential for UI in this critical period

(Elsheikh and Ahmed, 2004). This result is in consistent with the result of Sheldon et al. (2006) and Dolezel et al (2008) who reported that uterine bacterial infection during early PP delays UI. This study also confirms that, the initiation of the first follicular wave and the appearance of the first DF were affected by uterine bacterial infection during early PP. The dairy cows suffered severe PP uterine bacterial infection had a prolonged period for the initiation of the first follicular wave and the appearance of the first DF. These findings match with the findings of Huszenicza et al. (1999) who emphasized that dairy cows that suffered PP uterine bacterial infection had a prolonged time for initiation of the first follicular wave and the appearance of the first DF. The findings of this study also indicated that dairy cows which suffered severe uterine bacterial infection during early PP had a prolonged period for the recrudescence of the FO. This could be attributed to the delayed UI and the reduced pituitary FSH and LH surge (Dobson et al., 2000). This finding agrees with the finding of Opsomer *et al.*, (2000) who reported that dairy cows that suffered uterine bacterial infection during the first week PP had a delayed recrudescence of the FO. Consequently, the DO was extended, the rate of service per conception at subsequent breeding was increased and the CI was longer. These findings also match with the findings of several studies that recorded the effect of uterine bacterial infection during early PP on the DO, CI and the conception rate (Bartlett *et al.*, 1986; Huszenicza *et al.*, 1999).

In conclusion, the reduced fertility among the herd of the cross-bred dairy cows is presumably due to PP uterine infection. However, other factors can not be excluded.

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